

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
15 August 2002 (15.08.2002)(10) International Publication Number
WO 02/063042 A1(51) International Patent Classification⁷: C12Q 1/68,
C07H 21/00 [KR/KR]; 5-306 Hongsil Apt., Samsung-dong, Kangnam-gu, Seoul 135-090 (KR).

(21) International Application Number: PCT/KR01/00153 (74) Agents: KIM, Hak, Je et al.; Phoenix International, Kwangwhamoon, P.O. Box 1828, Seoul 110-618 (KR).

(22) International Filing Date: 3 February 2001 (03.02.2001)

(81) Designated States (national): AU, BR, CA, CN, ID, IL, IN, JP, KR, MX, NZ, RU, US, ZA.

(25) Filing Language: English

(84) Designated States (regional): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

(71) Applicant (for all designated States except US): BIONEX, INC. [KR/KR]; #731 College of Natural Science, Hangyang University, Haengdang-dong, Sungdong-gu, 17, Seoul 133-791 (KR).

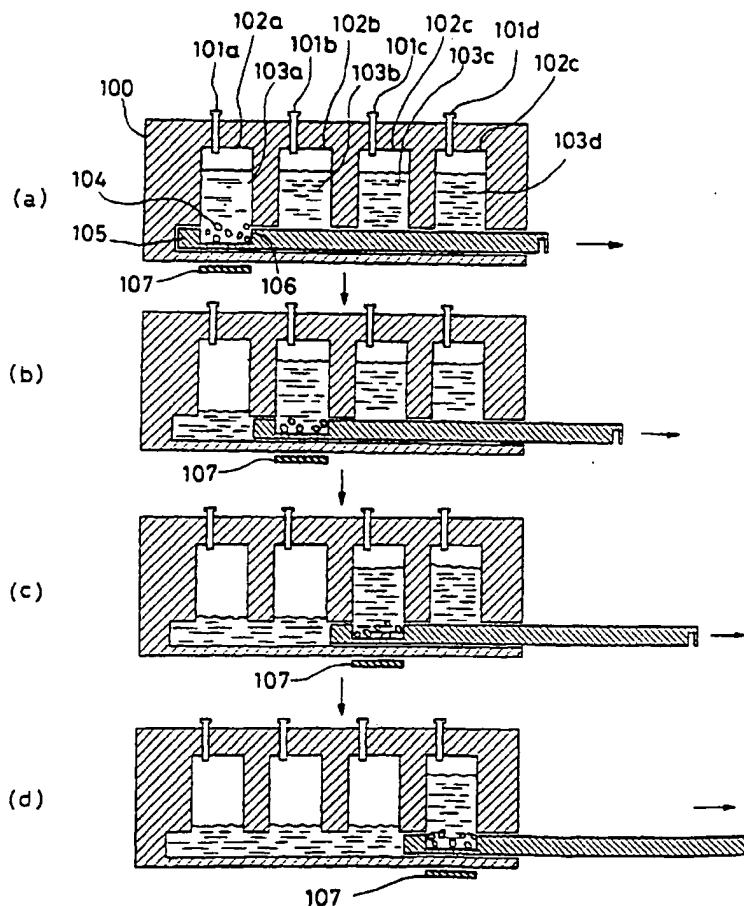
Published:
— with international search report

(72) Inventor; and

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(75) Inventor/Applicant (for US only): JANG, Gi, Young

(54) Title: APPARATUS FOR ISOLATING NUCLEIC ACID OR BIOLOGICAL MATERIAL



(57) Abstract: The present invention provides an apparatus for isolating nucleic acid or biological material from biological sample using a solid material that carries or absorbs nucleic acid or biological material, the apparatus comprising: a cassette in which plural chambers and input holes into the respective chambers are formed and the plural chambers are connected through a slot which is formed in the bottom of the chambers; a slider which is fitted through the slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the slider can be pulled back and forth according to the length of the slot; a cavity which is formed on the chamber side of the slider to settle or collect a solid material in the chamber; a means for pulling the slider to transfer the solid material from one chamber to the next chamber; and a means for agitating the liquid reactants and the solid material in the plural chambers.

WO 02/063042 A1

APPARATUS FOR ISOLATING NUCLEIC ACID OR BIOLOGICAL MATERIAL

FIELD OF THE INVENTION

The present invention relates to an apparatus for isolating and
5 purifying nucleic acid, cell and various biological materials.

BACKGROUND OF THE INVENTION

Isolation of nucleic acid or biological materials from various biological samples is important starting step in the fields of many areas such as biology, biochemistry, molecular biology, forensic medicine, 10 medical diagnostic and etc. Traditional method for isolating nucleic acid involves harmful organic solvents such as phenol and chloroform. (Sambrook, J., E. F. Fritsch and T. Maniatis 1989. Molecular cloning: A laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. N. Y.)

15 Recently, several methods were provided using materials that have the proclivity of binding nucleic acid. Concrete examples of these materials are silica (Boom, R., Sol, C. J. A., Salimans, M. M. M., Jansen, C. L., Wertheim-van Dillen, P. M. E., and van der Noordaa, J. (1990) J. Clin, Microbiol. 28, 495-503), glass fibers (Vogelstein, B., and Gillespie, 20 D. (1979) Proc. Natl. Acad. Sci. USA 76, 615-619, Marko, M. A., Chlperfield, R., and Birnbom, H. C. (1982) Anal. Biochem. 121, 382-

387), anion exchange resins (Wang, K., Gan, L., Boysen, C., and Hood, L. (1995) Anal. Biochem. 226, 85-90) and modified magnetic beads (Rudi, K., Kroken, M., Dahlberg, O. J., Deggerdal, A., Jakobsen, K. S., and Larsen, F. (1997) BioTechniques 22, 506-511, and Deggerdal, A.,
5 and Larsen, F. (1997) BioTechniques 22, 554-557).

The advantages of the method using these materials is that no harmful organic solvent is involved, and physical and biochemical degrading of nucleic acid during the isolation process is minimized. In addition, immobilized nucleic acid is less susceptible to digestion by
10 nucleic acid degrading enzymes.

The above mentioned methods, however, still need intensive manual pipetting steps to transfer the solid material to other vessel and thus the performer is vulnerable to potential viral and bacterial infection from infectious virus and bacteria if infected blood or bacteria is starting
15 material of nucleic acid isolation.

In order to avoid the intensive and tedious manual steps and to eliminate performer's potential error, several automatic machines such as "MagNa Pure LC" (Roche, Mannheim, Germany AG), "GENESIS" (Techan, Hombrechtikon, Switzerland) and others were developed for
20 high number of sample manipulation. Most of them apply magnetic bead to collect nucleic acid or biological materials from various biological samples to eliminate the use of harmful chemical solvents and centrifugation step. Although these machines are adequate to continuous isolation of nucleic acid or biological material and secure high

throughput, they are expensive, rather complicate and inefficient for low and medium number of sample manipulation so that they are not practical for most research and clinical laboratories.

In light of these drawbacks of the traditional apparatus for isolating
5 nucleic acid or biological material, there is a need for an apparatus that is efficient, inexpensive and reproducible as well as applicable to various biological samples.

SUMMARY OF THE INVENTION

A feature of the present invention is to provide an apparatus for
10 isolating a nucleic or biological material from low and medium number of biological samples in which intensive manual pipetting step is eliminated and the risk of viral and bacterial infections from the biological samples is reduced.

Another feature of the present invention is to provide an apparatus
15 for isolating nucleic acid or biological material from the biological samples less expensive and more readily available to researchers, medical personnel and other potential users of the technology.

According to the one aspect of the present invention, there is provided an apparatus for isolating nucleic acid or biological material
20 from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:

- (a) a cassette in which plural chambers and input holes into the respective chambers are formed and the plural chambers are

connected through a slot which is formed in the bottom of the chambers;

- 5 (b) a slider which is fitted through the slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the slider can be pulled back and forth according to the length of the slot,;
- (c) a cavity which is formed on the chamber side of the slider to settle or collect a solid material in the chamber;
- 10 (d) a means for pulling the slider to transfer the solid material from one chamber to the next chamber; and
- (e) a means for agitating the liquid reactants and the solid material in the plural chambers.

According to the another aspect of the present invention, there is provided an apparatus for isolating nucleic acid or biological material 15 from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:

- 20 (f) a cassette in which plural chambers and input holes into the respective chambers are formed, the bottoms of the plural chambers are connected through a first slot and the tops of the plural chambers are connected through a second slot;
- (g) a first slider which is fitted through the first slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the first slider can be pulled back and forth according to the length of the first slot,;

- (h) a cavity which is formed on the chamber side of the first slider to settle or collect a solid material in the chamber;
- 5 (i) a second slider having plural intruders toward the plural chamber to agitate the liquid reactants and the solid material, which is fitted through the second slot and can be pulled back and forth according to the length of the second slot;
- (j) a means for pulling the first slider to transfer the solid material from one chamber to the next chamber;
- 10 (k) a means for pulling the second slider to agitate the liquid reactants and the solid material; and
- (l) a means for agitating the liquid reactants and the solid material in the plural chambers.

In the present invention, depending on the kinds of solid material, the various biological samples can be applied over isolation of nucleic acid or biological materials. The various biological samples are blood, bacteria, cultured cells, polymerase chain Reaction (PCR) samples, DNA sequencing samples, DNA-containing agarose product and other biological reaction samples.

In the present invention, the examples of the solid material absorbing or binding nucleic acid or biological materials are silica, glass fiber, anion exchange resins, natural magnetic bead, modified magnetic bead, Sephadex, Sepharose and Sephacryl. Some solid material such as silica, glass fibers, anion exchange resins, Sephadex, Sephacryl and Sepharose have the binding ability to nucleic acid or biological

material and can be settled down somewhat rapidly under gravity. The other solid material such as natural or modified magnetic bead have the binding ability to nucleic acid or biological materials depending on modification on surface and affinity to magnet. The feature of the solid material allows the collection of the solid material into the cavity of the slider.

The advantages over the conventional isolation of nucleic acid or biological materials are:

- i) There is no need for tedious manual pipetting steps and therefore it is possible to eliminate the potential human error such as contamination and mishandling of the samples during the process;
- ii) The risk of infection by potential infectious agents inside the sample such as virus and bacteria is greatly reduced since the isolation procedure is done in a closed cassette; and
- iii) The number of samples analyzed at one time can be varied depending on the sets of chamber inside a cassette.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other object, feature, and advantage of the present invention will be apparent from the following detailed description of the preferred embodiments of the invention in conjunction with the accompanying drawings:

FIG. 1 is a cross sectional view illustrating sequential steps of isolating a nucleic or biological materials using an apparatus according

to the present invention;

FIG. 2 is a top view of an apparatus according to the present invention in which a cassette contains eight sets of plural chambers;

FIG. 3 is a cross sectional view of an apparatus according to one example of the present invention; and

FIG. 4 is a cross sectional view of an apparatus according to another example of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides an apparatus for isolating nucleic acid or biological material from various biological samples. The feature in the present invention is that solid material such as silica, glass fibers, exchange resin, modified magnetic beads and etc. is used for binding or absorbing nucleic acid or biological materials to remove the use of organic solvents and the centrifugation step. To eliminate the pipetting steps, a slider is applied for efficient movement of solid material binding nucleic acid or biological materials.

FIG. 1 is a cross sectional view illustrating sequential steps of isolating a nucleic or biological materials using an apparatus according to a preferred embodiment of the present invention.

As shown in **FIG. 1**, a cassette 100 comprises four chambers 102a, 102b, 102c and 102d with respective input holes 101a, 101b, 101c and 101d. The four chambers 102a, 102b, 102c and 102d are connected with one another with a slot which is fitted with a slider 105. Although the

slider 105 can be pulled back and forth according to the length of the slot, the slider 105 fits into the slot very tightly so that the spaces of the chambers 102a, 102b, 102c and 102d are separated and the liquid reactants 103a, 103b, 103c and 103d which is added into the chambers 5 102a, 102b, 102c and 102d are sustained respectively. The slider 105 has a small cavity 106 that is capable to settle or collect a solid material at one of its end parts. The depth, area and shape of the cavity can be varied depending on the kind of used solid material and the shape of the chambers 102a, 102b, 102c and 102d. Preferably, the cross sectional 10 shape of the cavity 106 is the same with that of the chambers 102a, 102b, 102c and 102d.

The liquid reactants 103a, 103b, 103c and 103d in each chambers 102a, 102b, 102c and 102d can be various buffers such as lysis buffer, ethanol elution buffer, distilled water and etc. which is needed in the 15 process of isolating nucleic acid or biological materials.

In case of isolating nucleic acid from blood using magnetic bead as solid material, the process starts with an addition of blood into the chamber 102a that already contains lysis buffer 103a and a magnetic bead 104. To facilitate the lysis reaction, the whole cassette 100 is 20 shaken, for example, back and forth, or in seesawing or rotating motion in conjunction with motioning system (not shown). After shaking of the cassette 100, a magnet 107 is applied to collect the DNA-carrying magnetic bead 104 into the cavity 106 of the slider 105 as shown in FIG. 1(a).

To transfer the magnetic bead 104 to the next chamber 102b, the slider 105 is pulled manually or by automatic pulling system driven by a motor (not shown) and the cavity 106 is located in the bottom of the chamber 102b as shown in FIG. 1(b).

5 After that, for washing the magnetic bead 104, the whole cassette 100 is shaken, for example, back and forth or in seesawing or rotating motion for agitating the liquid reactants 103b and the magnetic bead 104, and then the magnet 107 is applied to collect the solid material 104. The procedure of pulling the slider 105, shaking of the cassette 100 and 10 applying of the magnet 107 is repeated in the step of FIG. 1(c) and (d).

In FIG. 1(d), the liquid reactant 103d is an elution buffer to elute the nucleic acid from magnetic bead 104 and finally the liquid reactant 103d containing nucleic acid in the last chamber 102d is collected by pipetting through an input hole 101d.

15 Although the input hole 101a, 101b, 101c and 101d was shown up in a simplified format in FIG. 1, other input methods such as a self-sealing piercable diaphragm, and etc. can be used. Also the input holes 101a, 101b, 101c and 101d can be equipped with a sliding lid to avoid flowing of the liquid reactants 103a, 103b, 103c and 103d. In FIG. 1, the step of 20 magnet 107 application to collect the magnetic bead 104 can be omitted if the other type of solid materials such as silica and resins that are easily settled down by gravity is used instead of the magnetic bead 104.

In FIG. 2, a cassette 201 contains eight sets of the chambers 202 for isolating nucleic acid or biological materials from eight different

samples. A slider 205 can be pulled according to the direction of the arrow. Although the eight sets of the chambers are shown, the number of the chamber set can be varied from 1 to 20,000. The number of chamber set can be increased by preferably reducing the size of each chamber or 5 enlarging the size of the cassette. The shape of each chamber can be varied within limited area of the chamber.

FIG. 3 is a cross sectional view of an apparatus according to one embodiment of the present invention.

In the present embodiment, the apparatus is explained focused on a 10 means for pulling the slider, a means for generating magnetic force, and a means for agitating the liquid reactants and the solid material.

Agitation of the liquid reactants and the solid material is achieved by a seesawing part 311 that holds and seesaws the cassette.

The means for pulling the slider comprises a belt 314, a hook 313 15 that fixes the slider to the belt 314, a pivoting point 312 to hold one side of the belt 314, and a motor 315 that drives the belt 314. Accordingly, the slider is pulled by driving the motor 315.

The means for generating magnetic force comprises a magnet 318, a belt 317, a pivoting point 316 to hold one side of the belt 317 and a 20 motor 319 to drives the belt. Thereby, a magnet can be moved according to the movement of magnetic solid material.

Addition to that, a means for controlling the temperature of the plural chambers was added. The means for controlling the temperature is a heater 320 which is operated by electrical power and controlled by a

censor 321.

These four parts can be coordinated by programmable software to prosecute the isolation of nucleic acid or biological materials appropriately.

5 **FIG. 4** is a cross sectional view of an apparatus according to the other embodiment of the present invention. The main difference of the apparatus in **FIG. 4** from the apparatus in **FIG. 3** is addition of a second slider 408 on the upper part of a cassette 400 instead of seesawing part, and a system to pull the cassette body 400 and a cassette holder 408
10 instead of the system to pull a slider 405 and a magnet 407. To simplify the figure, the input holes are omitted. The second slider 408 is a flat plate having multi number of vertical intruder 410. The purpose of the second slider 408 is to agitate the liquid reactants and the solid material in the plural chambers. The second slider 408 has an advantage
15 especially for the case that the mixing reaction is well not achieved by shaking the cassette since the liquid reactants have high surface tension. The liquid reactants and the solid material are agitated by pulling the second slider 408 back and forth repeatedly. A means for pulling the second slider 408 comprises a belt (not shown), a hook 413 that fixes
20 the slider to the belt, and a motor (not shown) that drives the belt.

Movement of the solid material on the cavity is achieved by moving the cassette 400, the cassette holder 409, and the second slider 408 using a belt 411 connected to the cassette holder 409, and a motor 412 driving the belt 411.

What I claim is;

1. An apparatus for isolating nucleic acid or biological material from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:
 - 5 (a) a cassette in which plural chambers and input holes into the respective chambers are formed and the plural chambers are connected through a slot which is formed in the bottom of the chambers;
 - (b) a slider which is fitted through the slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the slider can be pulled back and forth according to the length of the slot;
 - (c) a cavity which is formed on the chamber side of the slider to settle or collect a solid material in the chamber;
 - 10 (d) a means for pulling the slider to transfer the solid material from one chamber to the next chamber; and
 - (e) a means for agitating the liquid reactants and the solid material in the plural chambers.
2. The apparatus according to the claim 1, wherein the slider is in a shape of flat plate in which the cavity is formed at one of its end parts.
- 20 3. The apparatus according to the claim 1, wherein the solid material is selected from the group consisting of silica, glass fiber, anion exchange resins, Sephadex, Sepharose, Sephacryl and magnetic bead.

4. The apparatus according to the claim 1, further comprising a means for generating magnetic force which is located below the slider to settle or collect a magnetic solid material.
5. The apparatus according to the claim 1, further comprising a means for controlling a temperature of the plural chambers..
6. The apparatus according to the claim 1, wherein the operation of the whole apparatus is automated.
7. The apparatus according to the claim 1, wherein the agitation of the liquid reactants and the solid material is achieved by shaking the cassette in the seesawing motion.
10
8. An apparatus for isolating nucleic acid or biological material from biological sample using a solid material which carries or absorbs nucleic acid or biological material, the apparatus comprising:
 - (a) a cassette in which plural chambers and input holes into the respective chambers are formed, the bottoms of the plural chambers are connected through a first slot and the tops of the plural chambers are connected through a second slot;
 - (b) a first slider which is fitted through the first slot to separate the spaces of the chambers, thereby the plural chambers contains a liquid reactant separately, and the first slider can be pulled back and forth according to the length of the first slot;
20
 - (c) a cavity which is formed on the chamber side of the first slider to settle or collect a solid material in the chamber;
 - (d) a second slider having plural intruders toward the plural

chambers to agitate the liquid reactants and the solid material, which is fitted through the second slot and can be pulled back and forth according to the length of the second slot;

- 5 (e) a means for pulling the first slider to transfer the solid material from one chamber to the next chamber;
- (f) a means for pulling the second slider to agitate the liquid reactants and the solid material; and
- (g) a means for agitating the liquid reactants and the solid material in the plural chambers.

10 9. The apparatus according to the claim 1, wherein the first slider is in a shape of flat plate in which the cavity is formed at one of its end parts.

10. The apparatus according to the claim 1, wherein the solid material is selected from the group consisting of silica, glass fiber, anion exchange resins, Sephadex, Sepharose, Sephacryl and magnetic bead.

11. The apparatus according to the claim 1, further comprising a means for generating magnetic force which is located below the slider to settle or collect a magnetic solid material.

12. The apparatus according to the claim 1, further comprising a means 20 for controlling a temperature of the plural chambers.

13. The apparatus according to the claim 1, wherein the operation of the whole apparatus is automated.

14. The apparatus according to the claim 1, wherein the agitation of the liquid reactants and the solid material is achieved by pulling back

and forth according to the length of the second slot.

FIG. 1

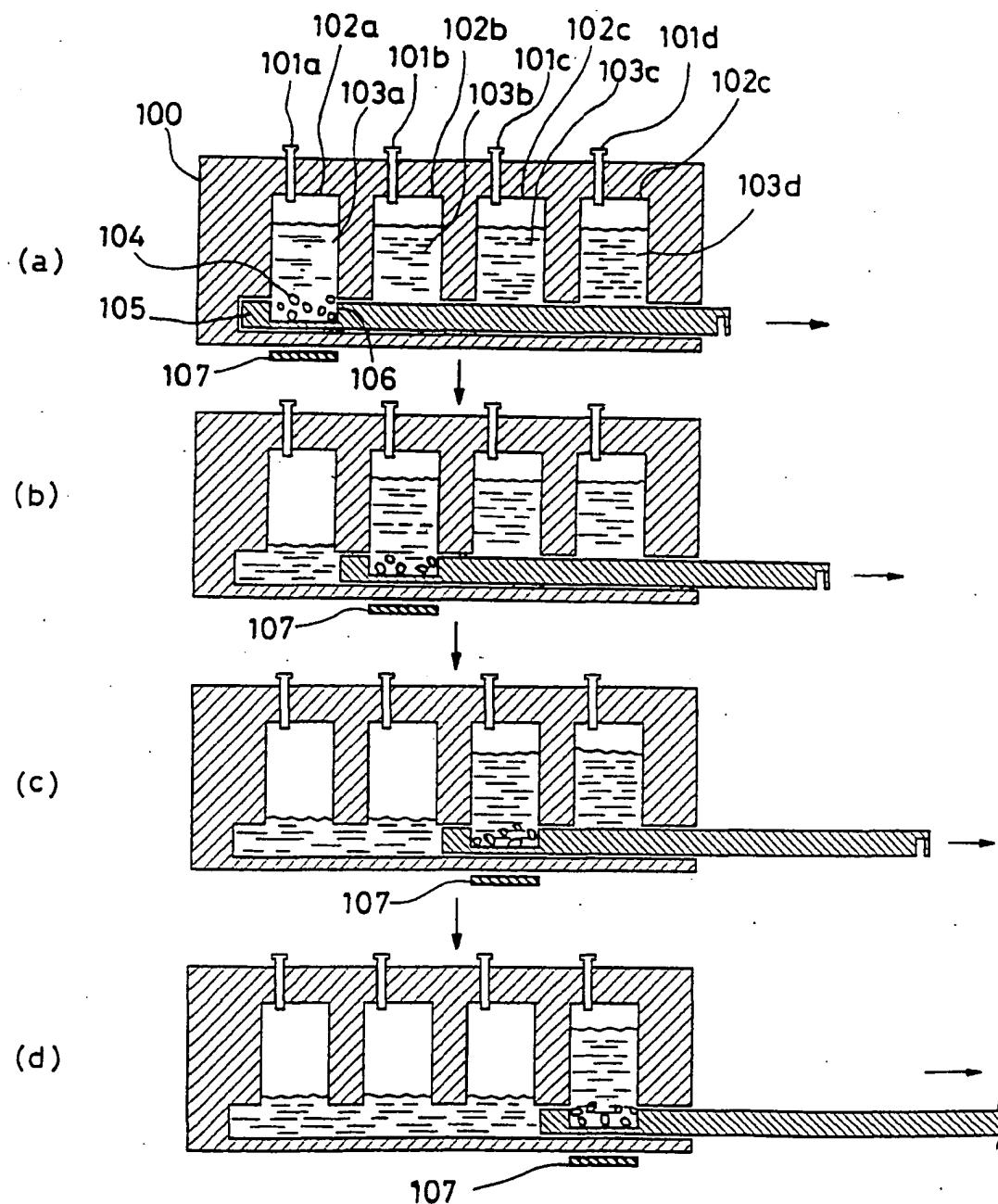


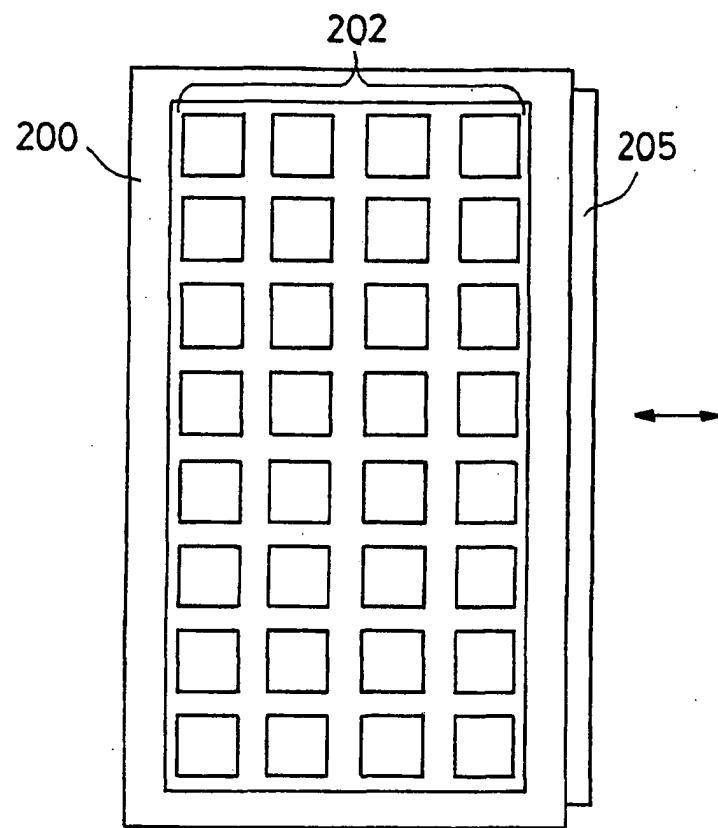
FIG. 2

FIG. 3

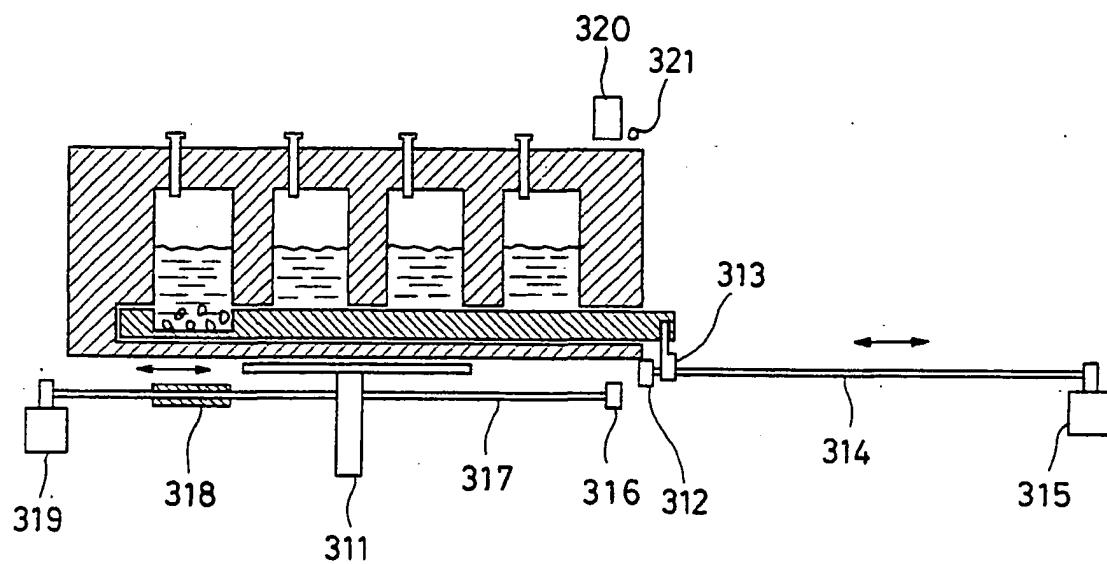
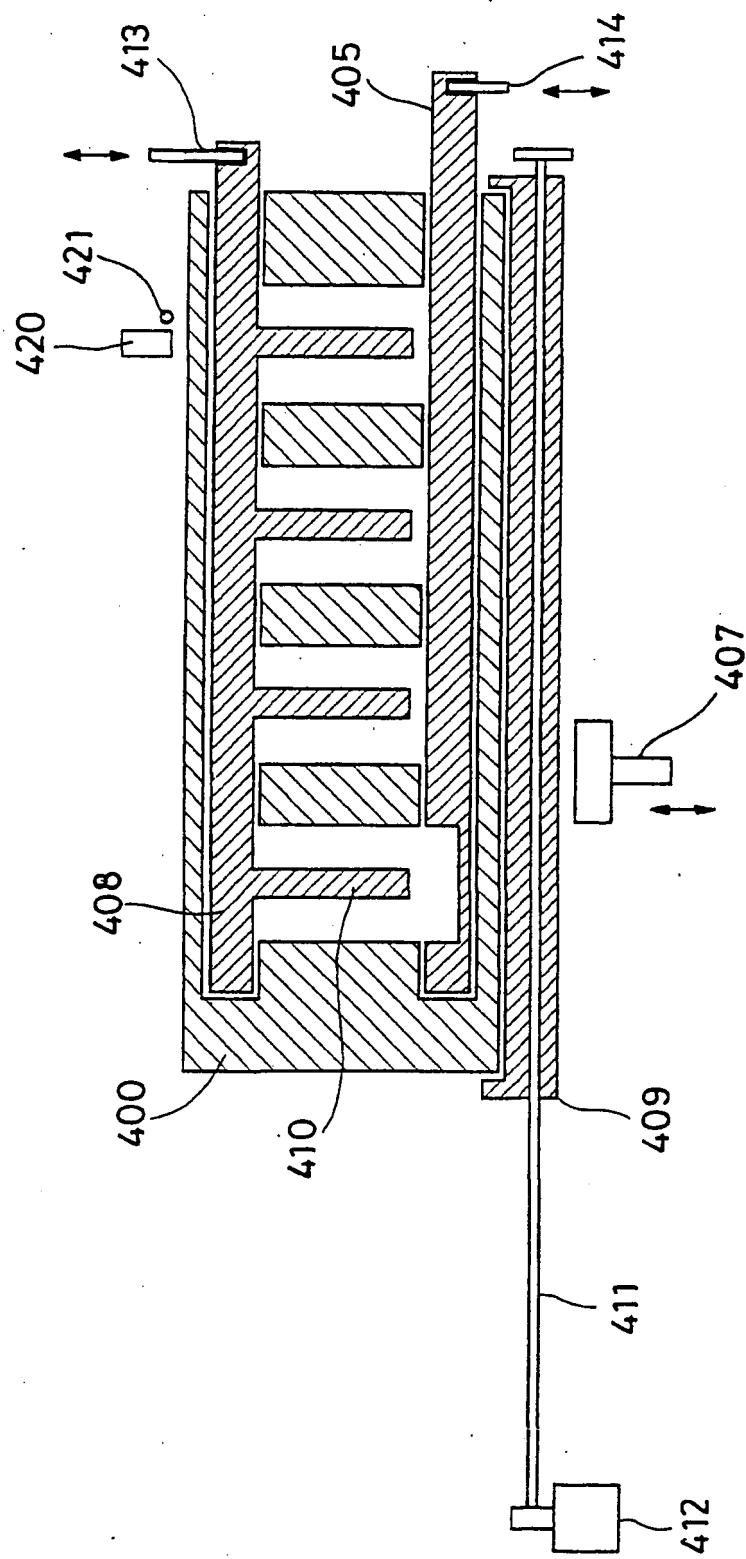


FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR01/00153

A. CLASSIFICATION OF SUBJECT MATTER**IPC7 C12Q 1/68, C07H 21/00**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C12Q 1/68, C07H 21/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

USPAT, FPD, PAJ "(apparatus or device) and (purif* or extract* or isolat*) and nucleic"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0487028 A2 (SHIMADZU CO.) 27 MAY 1992	1 - 14
A	WO 97/08547 A1 (THEOBALD SMITH RESEARCH INSTITUTE, INC) 06 MARCH 1997	1 - 14
A	EP 0933132 A2 (TOYO BOSEKI KABUSHIKI KAISHA) 04 AUGUST 1999	1 - 14

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 NOVEMBER 2001 (14.11.2001)

Date of mailing of the international search report

19 NOVEMBER 2001 (19.11.2001)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon
Metropolitan City 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

HAN, Hyun Sook

Telephone No. 82-42-481-5596



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR01/00153

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0487028 A2	27.05.92	JP4187077 A	03.07.92
WO 97/08547 A1	06.03.97	US 5804684 A AU 6857596 A	08.09.98 19.03.97